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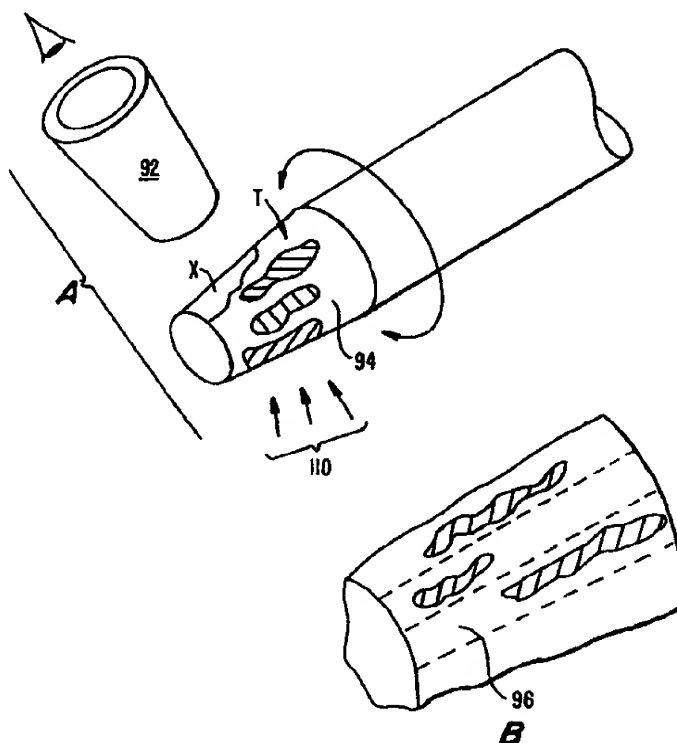
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US98/13318 <b>(22) International Filing Date:</b> 25 June 1998 (25.06.98) <b>(30) Priority Data:</b> 08/883,821      27 June 1997 (27.06.97)      US <b>(71) Applicant:</b> THE GOVERNMENT OF THE UNITED STATES OF AMERICA, [US/US]; represented by THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES, Bethesda, MD 20892 (US). <b>(72) Inventors:</b> GOLDSTEIN, Seth, R.; National Institute of Health, Building 13, Room 3N17, Bethesda, MD 20892 (US). BONNER, Robert, F.; National Institute of Health, Building 13, Room 3N17, Bethesda, MD 20892 (US). SMITH, Paul, D.; National Institute of Health, Building 13, Room 3N17, Bethesda, MD 20892 (US). PETERSON, John; National Institute of Health, Building 13, Room 3W12, Bethesda, MD 20892 (US). POHIDA, Thomas; National Institute of Health, Building 12A, Room 2033, Bethesda, MD 20892 (US). <b>(74) Agents:</b> HYNES, William, M. et al.; Townsend and Townsend and Crew LLP, 8th floor, Two Embarcadero Center, San Francisco, CA 94111 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>With amended claims.</i>

**(54) Title:** CONVEX GEOMETRY ADHESIVE FILM SYSTEM FOR LASER CAPTURE MICRODISSECTION

**(57) Abstract**

A tissue sample is conventionally visualized in a microscope. A selectively activated convex surface is provided, preferably at the distal end of a rod. This selectively activated convex surface when activated, typically with a laser through an optic light path in the microscope, provides the activated region with adhesive properties. At least one portion of the tissue sample which is to be extracted is identified. This identified portion is contacted with a portion of the selectively activated convex surface on the end of the rod. When the convex surface is activated, typically by exposure to laser light in the footprint of the desired sample, an adhesive transfer surface on the selectively activated convex surface is provided which adheres to the desired cells in the footprint of the desired sample. Thereafter, the adhesive transfer surface is separated from the remainder of the tissue sample while maintaining adhesion with the desired cells.



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## CONVEX GEOMETRY ADHESIVE FILM SYSTEM FOR LASER CAPTURE MICRODISSECTION

10           This disclosure deals with a convex geometry for the  
laser actuated adhesive film used to perform Laser Capture  
Microdissection (LCM) - a technique for removing minute  
amounts of tissue from pathology slides in a convenient, rapid  
manner. Several configurations for solving a variety of  
15           problems encountered in LCM are described, as well as a  
mechanical mechanism for performing some of the mechanical  
functions involved in the procedure.

### BACKGROUND OF THE INVENTION

20           Specifically, many diseases are now understood at the  
molecular and genetic level. Analysis of such molecules is  
important for disease diagnosis and prognosis. Current  
methods for direct extraction of cellular tissue material from  
a tissue sample are limited because the extraction reflects  
25           only the average content of disease associated markers. In  
reality, tissues are very heterogeneous and the most  
diagnostic portions of the tissue may be confined to a few  
hundred cells or less in a lesion. Thus molecular analysis of  
human pathology sections will require the targeting and  
30           removal of pure populations of homogeneous cells from within a  
specimen where these cells may comprise only a few percent of  
the total local tissue or even much less (e.g., 1 cell in a  
1000 or even less). The modification of the LCM invention  
covered in this application is particularly important in the  
35           collection of sparse or rare cells within a tissue and cases  
where such cells must be collected from a variety of specimens  
in order to provide sufficient material for an accurate  
statistical representation of the pathology. Examples include  
isolation cells that were infected by an AIDS virus,  
40           macrophages containing an infectious tuberculosis bacteria,  
cells within a kidney glomerulus in patients exhibiting  
proteinuria, brain cells and plaques in brain exhibiting

Alzheimer's Syndrome, as well as cancerous tissues. In each case the precise molecular description of the variant of the disease which might be related to different natural etiology and responses of that particular patient to different therapies can only be accomplished without gross contamination of molecules from surrounding cells. Though we specifically discuss how laser microdissection is critical in the molecular analysis of cancer, the same principles apply to a molecular description (e.g., DNA mutations, alterations of gene expression, and post-transcriptional modification of proteins) of a variety of diseases and their response to drug treatment and of normal human developing and aging.

Normal tissue samples contain a variety of cell types surrounding and adjacent to the pre-invasive and invasive tumor cells. A region of the tumor tissue subject to biopsy and diagnosis as small as 1.0 mm can contain normal epithelium, pre-invasive stages of carcinoma, in-situ carcinoma, invasive carcinoma, and inflammatory areas. Consequently, routine scraping and cutting methods will gather all of these types of cells, and hence, loss of an allele will be masked by presence of a normal copy of the allele in the contaminating non-malignant cells. Existing methods for cutting away or masking a portion of tissue do not have the needed resolution. Hence the analysis of genetic results by those previous methods are always plagued by contaminating alleles from normal cells, undesired cells or vascular cells.

The molecular study of human tumors is currently limited by the techniques and model systems available for their characterization. Studies to quantitatively or qualitatively assess proteins or nucleic acid expression in human tumor cells are compromised by the diverse cell populations present in bulk tumor specimens. Histologic fields of invasive tumor typically show a number of cell types including tumor cells, stromal cells, endothelial cells, normal epithelial cells and inflammatory cells. Since the tumor cells are often a relatively small percentage of the total cell population it is difficult to interpret the significance of net protein or nucleic acid alterations in these specimens.

Studies of human tumor cells in culture do not account for the complex interactions of the tumor cells with host cells and extracellular matrix, and how they may regulate tumor cell protease productivity or activation.

5 Immunohistochemical staining allows one to examine enzyme distribution in regions of tumor invasion, however, results vary with tissue fixation and antibody-antigen affinity, and provide only a semi-quantitative assessment of protein levels. Furthermore, quantitative interpretation of staining results  
10 is complicated by the variability of staining patterns within tissue sections, subjective evaluation of staining intensity, and the difficulty in interpreting the significance of stromal staining. In addition, many antibodies utilized in the study of proteases do not differentiate pro-enzyme from active  
15 enzyme species. Assays of enzyme or mRNA levels from homogenates of human tumors does not account for either the mixed population of cells within the specimens, or the concomitant pathophysiologic processes which may occur in the tissue.

20 Prior methods of study have not allowed investigators to specifically examine genetic alterations in pre-invasive lesions. Even the most sophisticated genetic testing techniques to date have been of limited value because the input DNA, RNA or proteins to be analyzed are not derived from  
25 pure cell populations exhibiting the disease morphology. Several methods have been reported for tissue microdissection to address this problem. These include gross dissection of frozen tissue blocks to enrich for specific cell populations, irradiation of manually ink stained sections to destroy  
30 unwanted genetic material, touch preparations of frozen tissue specimens and microdissection with manual tools. These methods, however, are not sufficiently precise and efficient for routine research or high throughput clinical molecular diagnostic applications. Manual microdissection, for example,  
35 has good precision but is time consuming, labor intensive, requires a high degree of manual dexterity, and is not generally suitable for the ordinary technologist.

In Lance A. Liotta et al. US Provisional Patent Application Serial 60/036,927 filed February 7, 1997, entitled Isolation of Cellular Material Under Microscope Visualization, there is described a technique that we have come to call Laser Capture Microdissection (LCM). Simply stated, a method and apparatus was disclosed in which a tissue sample was provided, typically on a slide under observation in a microscope. The tissue was contacted with a selectively activated surface which could be activated to provide selective regions thereof with adhesive properties. The tissue sample is visualized through a microscope and at least one portion of the tissue sample which is to be extracted is identified. Thereafter, the selectively activated surface is activated, typically by a laser routed through a fiber optic being directed onto the selectively activated surface in the footprint of the desired tissue. This is done while a region of selectively activated surface is in contact with the portion of the tissue sample selected. The activated region of the selectively activated surface adheres to that portion of the tissue sample. Thereafter, the activated surface is separated from the tissue sample while maintaining adhesion between the activated region of selectively activated surface and the portion of the tissue sample. The portion of the tissue sample is extracted from the remaining portion of said tissue sample.

While the basic technique disclosed in the above-entitled patent deals with applying a large free piece of EVA film to a tissue sample on a slide, the reduction of this technique to a practical method and apparatus which can be utilized by relatively untrained personnel with conventional microscopes is not set forth. Accordingly, in the following specification, one practical embodiment of this technique is set forth.

#### SUMMARY OF THE INVENTION

A tissue sample is conventionally visualized in a microscope. A selectively activated convex surface is provided, preferably at the distal end of a rod. This selectively activated convex surface when activated, typically

with a laser through an optic light path in the microscope, provides the activated region with adhesive properties. At least one portion of the tissue sample which is to be extracted is identified. This identified portion is contacted with a portion of the selectively activated convex surface on the end of the rod. When the convex surface is activated, typically by exposure to laser light in the footprint of the desired sample, an adhesive transfer surface on the selectively activated convex surface is provided which adheres to the desired cells in the footprint of the desired sample. Thereafter, the adhesive transfer surface is separated from the remainder of the tissue sample while maintaining adhesion with the desired cells. Thus the desired portion of the tissue sample is extracted. The disclosed selectively activated convex surface is preferably utilized to collect desired tissue samples at more than one location on the same slide or from different slides. The collected tissue samples can thereafter be inspected if desired, as collected on the convex surface, and then liberated - as by dissolving the proteins of the samples. This can effectively concentrate occurring remaining cells in order to obtain enough pure material for analysis. A rod having a convex surface with the selectively activated material is set forth as a staple for use with the apparatus and process. Preferred shapes for the convex surface are disclosed as well as a method for coating rods with a resultant rod article.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a side elevation view of a conventional inverted microscope having the convex geometry adhesive film system of this invention illustrated in solid lines for the collection of a sample by laser capture microdissection and illustrating in broken lines the pivoted position of the apparatus to permit convenient loading and unloading of a new rod out of the optical path;

Fig. 2 is an exploded view of the mechanisms of the convex geometry adhesive film system of this invention without attachment to a microscope;



Fig. 3 is a view of two typical slides side-by-side each of which contains tissue samples illustrating cell locations on the slides remote from one another for collection by the convex geometry adhesive film system;

5 Fig. 4A is a perspective view of a frustum-shaped surface having the desired cells collected on the selectively activated film on the frustum surface, with the view schematically showing inspection of the collected samples by the microscope;

10 Fig. 4B is a magnified view of the collection surface of Fig. 4A illustrating the large numbers of samples that can be collected upon a single convex surface;

15 Fig. 5A - 5E is a series of illustrations setting forth possible convex geometries that can be placed on the sample with,

Fig. 5A illustrating a cylinder,

Fig. 5B illustrating a portion of an oblate spheroid,

Fig. 5C illustrating a portion of a sphere,

20 Fig. 5D illustrating a portion of a sphere having facets,

Fig. 5E illustrating a frustum having facets,

Figs. 6A and 6B illustrate dipping and drying of a rod,

25 Fig. 7 illustrates spray coating of a rod, and

Fig. 8 illustrates application of a measured coating to a rod.

#### DESCRIPTION OF THE PREFERRED EMBODIMENT

30 Referring to Fig. 1, sample collector C is shown as an attachment to inverted microscope  $M_1$ . As is conventional, inverted microscope  $M_1$  includes eyepiece E, objective O, conventional light source L all assisting in observing slide S having sample A.

35 Sample collector C is an attachment, only schematically shown in Fig. 1. It includes sample collecting rod R which is pivotal about vertical pivot axis V. Sample collecting rod R

has coated collection tip T which is at the site on sample A of the desired cells to be collected.

Light Z from a laser enters the microscope through the epi-illumination port and is focused on the slide at a known, predetermined point, e.g. the center of the field of view.

In order to conveniently replace and remove the replenishable part of R, sample collecting rod R of sample collector C can be pivoted about axis V to move it out of the objective O to eyepiece E light path as shown by sample collecting rod R' in broken lines. This system will be more completely described with respect to Fig. 2.

Operation can be simply described. First the microscope stage is centered and the sample collecting rod R is placed in position above the slide. Next, slide S is inspected until desired cells, sample A are located. The slide is positioned on the stage so that sample A is at the center of the field. Then, sample collecting rod R having coated collection tip T is lowered by being pivoted about a horizontal axis using a mechanism that will be later explained in Fig. 2, so that contact occurs between collection tip T and sample A at the desired cells. Then, laser Z activates coated collection tip T and causes adhesion at the desired cells. If desired, small motions of the microscope stage can be used to position extended regions of A and the collection tip T in the laser beam in order to collect more than a single point of tissue of A corresponding to one laser spot. When all parts of the combination of A and tip T have been exposed to the laser, coated collection tip T with the desired cells adhered to it is then lifted off sample A, sample collecting rod R is rotated about its axis - only slightly - and the entire above process is repeated with a different sample, A<sup>1</sup>.

Having grossly summarized the operation, description of the preferred embodiment will continue.

Referring to Fig. 2, sample collector C is shown together with part of sample collecting rod R. Stage mounting 14 mounts at split ring mounting 16 to main bushing 18. Main bushing 18 supports bearing block 20. Bearing block 20 in

turn supports sample collector base 22. Vertical pivot axis V fits into bearing block 20 at bearing block central aperture 24 and is locked against relative rotation.

Detent block 26 locks to vertical pivot axis V and supports spring mounted detents 28. Locking of detent block 26 to vertical pivot axis V occurs at detent shaft lock 30.

Sample collector base 22 defines slide registering detent groove 32 and remote location detent groove 34. Simply stated, when sample collector base 22 is registered with spring mounted detents 28 overlying slide registering detent groove 32, sample collecting rod R with coated collection tip T overlies the central view point of sample A on slide S. When the stage is centered this central view point coincides with the location of the laser beam. When sample collector base 22 is registered with spring mounted detents 28 overlying remote location detent groove 34, sample collecting rod R at coated collection tip T is away from slide S and its sample A permitting convenient loading and unloading of the replenishable part of rod R away from the slide S. (See Fig. 1.)

Sample collecting rod R as shown in Fig. 2 includes at its distal end coupling 40 for connection to various sequential sample collecting replenishable rod tips each with their own coated collection tip T. Such tips will be discussed later with respect to Figs. 5A - 5E. This same sample collecting rod R at the opposite end connects to rotational stepper motor 42. After each sample is collected on coated collection tip T, rotation by a small fraction of a full turn of rotational stepper motor 42 occurs until coated collection tip T has samples around the entire periphery (or less, if desired).

To avoid shearing the tissue and causing possible contamination, it is necessary that sample collecting rod R at coated collection tip T come out of contact with sample A on slide S before it is rotated. Accordingly, rotational stepper motor 42 is mounted to pivot block 44. Pivot block 44 in turn rocks about horizontal pivot shaft 46 within pivot aperture 48 at the end of sample collector base 22. Thus it will be seen

that sample collecting rod R at coated collection tip T can move into and out of contact with sample A on slide S. After the rod has been rotated, the stage recentered, and the slide moved to a new position with desired cells at the center of the field, the rod is lowered.

The contact of coated collection tip T with sample A on slide S must be precisely controlled. This is done by using a leaf spring actuator to control the amount of force utilized for each contact of the sample collecting rod R.

This controlled contact of coated collection tip T at the end of sample collecting rod R is determined by vertical stepper motor 54 which attaches underneath sample collector base 22. Motor 54 having vertically extending stepper shaft 56 which upon motor actuation undergoes a linear vertical excursion. Vertically extending stepper shaft 56 fastens at the leaf spring 60 end adjacent linear expansion curve 62. At the opposite end, leaf spring 60 has pivot block bolt 65 which attaches to pivot block 44 at pivot block bolt aperture 67.

Having set forth this structure, operation of sample collector C to pivot sample collecting rod R at coated collection tip T into and out of contact with sample A on slide S can be easily understood. Specifically, vertical stepper motor 54 is moved in vertical excursion a controlled (e.g., computer) amount to carefully adjust the height of vertically extending stepper shaft 56. Stepper shaft 56 causes leaf spring 60 to tilt pivot block bolt 65. Pivot block bolt in turn pivots pivot block 44 and mounted rotational stepper motor 42 with attached sample collecting rod R. As rod R has coated collection tip T at the distal end, controlled touching of sample A can occur.

It will be understood that the disclosed mechanism is extremely sensitive. Given the long lever arm of leaf spring 60, precision control over the force on sample A is easily achieved. Further, the amount of force utilized for the contact can control, within limits, the width of the "line" contact area of sample collected. Contact with reduced force will collect reduced amounts of sample A; contact with increased force will collect increased amounts of sample A.

In the positioning of desired cells of sample A on slide S for collection, two movements can be utilized. First, referring to Fig. 1, slide S can be provided with gross motion relative to conventional microscope stage S so as to center the sample A in the field of view. This sliding motion is well known to anyone who has operated a microscope.

Second, some minute movement of the conventional microscope stage 70 is desired for collecting desired cells located anywhere within the contact zone of R.

An alternative to raising and then rotating and then lowering the rod R is to roll the rod over the area A in conjunction with controlled motion of the stage so there is no shearing of the tissue.

An alternative to small stage motions is to scan the laser beam, turning it on and off so that only the desired cells and the adjacent adhesive are illuminated by the laser.

Having described sample collector C with sample collecting rod R and coated collection tip T, the operation of the tip with respect to the sample can now be set forth.

To evaluate some performance parameters for the cylindrical geometry concept, it is convenient to use the following parabolic approximation of a circle, good for small distances x:

$$y = (x^2) / 2R$$

This approximates a circle of radius R whose center is at  $y=R$  and  $x=0$  (e.g. its lowest point is at the origin which is assumed to be the point of contact between the rod/film and the tissue). If  $2R = 3/16$  inch ( $= 4.68\text{mm}$ ), then the circumference is 14.7 mm. In order for y to lift off by 50 microns (.002 inches) to avoid contamination with the tissue at the next transfer site,  $x=0.48\text{mm}$  using the above relation. This would allow 30 transfers on a single rod assuming the entire periphery is used and the transfers are spaced 0.48mm apart on centers. For visualization, if one allowed a maximum Y of 1 micron to maintain focus on the tissue while it is on the curved surface, the resulting  $X = .070\text{mm}$ . Thus a field of  $\approx 140$  microns wide will be in sharp focus for subsequent viewing without the need to refocus onto a curved surface.

The question arises whether the contact area will be large enough to be useful. One can make a crude estimate of the contact area using the classical Hertzian contact stress equations which apply to an elastic cylinder of radius R and length L and modulus E in "line" contact with an elastic semi-infinite medium having a plane surface. Assuming that the properties of the latter are given by those of the glass slide (and not the ~7 micron thick tissue) the properties of the plastic dominate and the relevant parameter is approximately  $1/E$ .

The contact pressure  $p$  is given by  $p = p_0(1 - y^2/b^2)^{1/2}$  where  $p_0 \approx 1/2(PE/RL)^{1/2}$ . The contact semi-width  $b$  is given by  $b \approx (PR/EL)^{1/2}$ . Using values of  $2R = 3/16$  inch,  $L = 1$  mm,  $P = 1$  lb, and  $E = 10^5$  psi,  $b$  is 125 microns, and  $p_0$  is 2500 psi. If  $P = 1$  oz, then  $b = 30$  microns, and  $p_0 = 625$  psi. If  $E$  is  $10^4$  psi (a possibility), then  $b \approx 400$  microns and  $p_0 \approx 800$  psi for  $P = 1$  lb, and  $b = 100$  microns and  $p_0 = 200$  psi for  $P = 1$  oz.

Thus it appears as if the "line" contact area will be large enough to be useful and small enough so that adjacent samples on the periphery can be kept separate. However, these calculations are to be taken with care since the EVA film used for the preferred coating may deform plastically in cases of high contact stress which would tend to increase the contact area, as would taking the tissue properties into account. On the other hand the compliant elastic is only in a thin film on a relatively rigid cylinder which would tend to make the contact area smaller. Our experiments indicate that with reasonable contact forces, the desired area can be obtained.

Since the theoretical stress distribution is parabolic (which will be smoothed out by deviations from the ideal Hertzian model), the question arises as to whether the stress can be controlled well enough so that it allows good transfer but does not cause nonspecific pickup. (This situation is no worse than the existing situation with flat geometries - it is just more explicit since it is easier to "analyze".) Once again, our experiments indicate that this is possible. We frequently use a contact force of 1 gm which leads to a maximum pressure  $p_0$  of ~40 psi and a  $b$  of ~20  $\mu$ .

The situation is simpler with the polygonal cross section rods. If a facet is 1 mm long in the peripheral direction and 1 mm wide in the rod axial direction, then the contact area is  $1/625$  inches<sup>2</sup>. If the contact force is 1 oz and the contact stress is assumed to be uniform (due to the flat surface), then its value is  $1/16$  lb/ $1/625$  in<sup>2</sup> = 40 lb/in<sup>2</sup> psi, a value that is known to give good results.

The issue of alignment of the contact surface with the tissue sample is relevant. Assume that the rod is relieved so that only a 1 mm length of it (where the film is deposited) is in contact with the tissue. Assume that the mechanical mechanism has been adjusted so that everything is perfectly aligned i.e., the contact surface is exactly parallel to the slide when it has been lowered to touch the slide so that the gap between the two uniformly vanishes. In a preliminary design, the relevant pivot point for the lift off of the rod is approximately 4 inches from the contact point with the tissue. Even if the pivot point shifts 0.005 inches vertically due to operation of the mechanism, (unlikely in a reasonably good mechanism), the change in the contact angle will only be  $\sim .00125$  radians. Taken over the 1 mm (1000 micron) length of the film contact area, this minute change in contact angle causes only slightly more than 1 micron change in elevation of the film off the tissue. This is within the anticipated nonuniformities in the film and tissue and would not have a significant effect.

Having set forth the physics related to the contact of coated collection tip T on sample collecting rod R with sample A on slide S, some attention can be devoted to the particular configurations which could be used at coated collection tip T at the end of sample collecting rod R.

Referring to Fig. 3, the overall problems to which the disclosure is directed can be discussed. Slide S<sub>1</sub> having sample A<sub>1</sub> is shown along side slide S<sub>2</sub> having sample A<sub>2</sub>. Examining the illustrated (and over-simplified illustrated samples) desired cells 88 are shown interspersed with undesired cells 90. The reader will understand that the real biological world is vastly more complex than this

illustration. However that may be, using these slides and referring to the collection apparatus and method here set forth, three important observations about this disclosure can be made.

5 First, where desired cells 88 are collected, the problem is frequently collecting sufficient amounts of the desired cells for further analysis. In the illustrated slides, it is to be understood that collection of one of the three groups of desired cells 88 may produce insufficient quantity for further  
10 testing. Further, observing both slides, the cells present on one slide may not be sufficient; collection of cells from a second sample  $A_2$  on a second slide  $S_2$  or additional slides may be required. Thus, a single collection tip T can effectively concentrate many rarely occurring cells from a large amount of  
15 histological tissue. Further, collection on more than one coated collection tip T can occur. Simply stated, the process of collection of desired cells 88 can continue until the desired amount is present and ready for testing.

20 Secondly, referring to Figs. 4A and 4B, it will be understood that the convex surface concept here disclosed is an ideal way to collect and concentrate such cells. Referring to Fig. 4A, an exaggerated collection of desired cells 88 on coated collection tip T is shown. Referring to Fig. 4B, a denser concentration of desired cells 88 is shown. In actual  
25 fact, it is contemplated that typically 30 such samples can be collected around the periphery of the coated collection tip T. This should assure collection of sufficient sample for further testing.

30 Third, once the desired cells 88 are collected on coated collection tip T, they may thereafter be inspected before further processing. For example, a pathologist may not want to undertake the tedium (albeit reduced tedium) required for the cell collection. At the same time, that pathologist knows the exact nature of desired cells 88 for collection. Once a  
35 technician has collected the cells, inspection of the cells on coated collection tip T can be performed by a pathologist or other technical person. This would typically occur under an



inverted microscope  $M_1$  - but is schematically shown here at eye loop 92.

It may well be that certain collected specimens may fail to pass such inspection. These specimens can be prevented from subsequent processing either by precision encapsulation using the laser to remelt the adhesive, or by destruction through irradiation e.g., UV to destroy DNA or an intense focal heat pulse with a laser. This is illustrated by post attachment treatment 110 in Fig. 4A.

It will be understood that liberation of at least part of desired cells 88 can occur by many convenient expedients. For example, dissolving the protein present in the cells can liberate DNA and RNA present for further testing.

Confining attention to coated collection tip T shown in Fig. 4A, a general discussion can be made. Coated collection tip T includes an underlying convex surface X. Overlying convex surface X, coating 94 is shown. Some general discussion about coating 94 is in order.

First, coating 94 must be capable of activation to produce the desired adhesion qualities desired. Second, coating 94 must be capable of adhering to convex surface X. Third, it is desirable that sample collecting rod R at convex surface X be transparent. This enables conventional transmission observation of desired cells 88 of sample A during all stages of the collection procedures.

Thermoplastic polymer films are widely used as heat-and pressure-activated adhesives for bonding surfaces. Most of these polymer films are transparent or translucent to visible light used in conventional light microscopy. These films are, however, strongly absorptive in specific regions of the electromagnetic spectrum (e.g., in regions of the infrared associated with strong molecular vibration modes such as 3000, 1800, 1400-960  $\text{cm}^{-1}$ ).

The activatable adhesive film 94 may be made of a wide variety of electromagnetically or thermally activatable materials, such as ethylene vinyl acetate (EVA), polyurethanes, polyvinyl acetates, and the like. Specific other selectively activatable materials found useful in the

practice of the invention are: thermal sensitive adhesives and waxes, such as Precision Coatings product #HAL-2 180C; thermally-activated hot glues and sealants, such as those from Ban Fastening Systems (Brooklyn, NY); ultraviolet sensitive or curing optical adhesives, such as ThorLabs, Inc. product N060-N0A81; thermal or optical emulsions, such as silkscreen coated emulsion B6, high mesh powdered, reconstituted left fixit emulsion (Riso Kagaku Corp.) and various other compounds including acetal, acrylic, alloys and blends, allyl, bismaleimides, cellulose, epoxy, fluoroplastics, ketone-based resins, liquid crystal polymers, melamine-formaldehyde, nitrile, nylon, phenolic, polyamide, polyacrylate, polybenzimidazole, polybutylene, polycarbonate, thermoplastic polyester, liquid crystal polymers, polybutylene terephthalate (PBT), polycyclohexylenedimethylene terephthalate (PCT), engineering grade polyethylene terephthalate (PET), standard grade polyethylene terephthalate (PET), thermoset polyetherimide polyethylene polyester, branched polyethylene, ethylene acid copolymer, ethylene-ethyl acrylate (EEA), ethylene-methyl acrylate (EMAC), ethylene-vinyl alcohol copolymers (EVOH), high-density polyethylene, HMW-high-density polyethylene, Ionomer, linear low-density polyethylene, linear polyethylene, low-density polyethylene, UHMW polyethylene, very-low-density polyethylene, thermoplastic polyimide, thermoset polyimide, polymethylpentene, modified polyphenylene oxide, polyphenylene sulfide, blow molding PPS, polyphthalamide, polypropylene, polypropylene homopolymer, polypropylene impact copolymers, polypropylene random copolymers, silicones, styrenic resins, ABS, ACS, acrylic-styrene-acrylonitrile, expandable polystyrene, general purpose polystyrene, impact polystyrene, olefin-modified SAN, polystyrene, styrene-acrylonitrile (SAN) and styrene-butadiene copolymers.

It is also possible to add infrared absorbing dyes to the thermoplastic films 94 to provide strong absorption at other specific infrared wavelengths without altering the films transparency to visible light. Such dyes are preferably IR absorbing dyes which are readily soluble in the plastic films

and have a very strong, narrow IR or near-IR absorption bands that can be matched to a variety of IR or near-IR lasers (including particularly laser diodes). If the focused pulse of electromagnetic radiation (e.g., laser) is delivered at wavelengths that are strongly absorbed by the film, then the film may be efficiently focally heated.

Many dye types could be considered for IR absorption, since most classes of visible absorbing dyes can be extended in wavelength by molecular modification. Phthalocyanines and cyanines have been among the most popular dyes because of stability, ease of preparation, solubility, optical and other properties. Moreover, the number of possible modifications of these dyes is very large because various central metal atoms which can be added and a variety of ring attachments which can be made to them. A book which gives a general overview of IR absorbing dyes is:

INFRARED ABSORBING DYES

Masaru Matsuoka, ed. (U. of Osaka, Sakai, Osaka)  
Plenum Press NY 1990

0-30843478-4

TA1690.I53 1990 available in the NBS library  
series: Topics in Applied Chemistry, A.R. Katritzky  
and G.J. Sabong, eds.

As an example of phthalocyanine dyes, the following 60 entries are in the Aldrich Chemical Catalog:

TABLE 1: PHTHALOCYANINE DYES (Aldrich Chemical Company)

#412066	Name:	TETRAKIS(4-CUMYLPHENOXY)PHTHALOCYANINE, 97%
#404543	Name:	TIN(II) PHTHALOCYANINE
#406481	Name:	SILICON PHTHALOCYANINE DIHYDROXIDE Cata
#414387	Name:	VANADYL 2,9,16,23-TETRAPHENOXY-29H,31H-PHTHALOCYANINE
#393932	Name:	MANGANESE(III) PHTHALOCYANINE CHLORIDE
#410160	Name:	IRON(II) PHTHALOCYANINE BIS(PYRIDINE) COMPLEX
#404551	Name:	TITANYL PHTHALOCYANINE
#418145	Name:	1,8,15,22-TETRAPHENOXY-29H,31H-PHTHALOCYANINE
#418153	Name:	2,9,16,23-TETRAPHENOXY-29H,31H-PHTHALOCYANINE
#379573	Name:	IRON(III) PHTHALOCYANINE CHLORIDE Catal
#406473	Name:	TIN(IV) PHTHALOCYANINE DICHLORIDE Catal
#415448	Name:	NICKEL(II) TETRAKIS(4-CUMYLPHENOXY)PHTHALOCYANINE
#418161	Name:	1,8,15,22-TETRAKIS(PHENYLTHIO)-29H,31H-PHTHALOCYANINE
#418188	Name:	2,9,16,23-TETRAKIS(PHENYLTHIO)-29H,31H-PHTHALOCYANINE
#408808	Name:	GALLIUM(III) PHTHALOCYANINE CHLORIDE Ca

#418986 Name: ALUMINUM 2,9,16,23-TETRAPHENOXY-29H,31H-PHTHALOCYANIN  
 #310204 Name: COPPER(II) 4,4',4'',4'''-TETRAAZA-29H,31H-PHTHALOCYAN  
 5 #402737 Name: MAGNESIUM PHTHALOCYANINE  
 #402745 Name: DISODIUM PHTHALOCYANINE  
 #418250 Name: ALUMINUM 2,9,1  
 #341169 Name: ZINC PHTHALOCYANINE  
 #379557 Name: MANGANESE(II) PHTHALOCYANINE Catalog Nu  
 10 #414379 Name: NICKEL(II) 2,9,16,23-TETRAPHENOXY-29H,31H-PHTHALOCYAN  
 #433462 Name: METHYLSILICON(  
 #418234 Name: ZINC 2,9,16,23-TETRAKIS(PHENYLTHIO)-29H,31H-PHTHALOCY  
 15 #418242 Name: ALUMINUM 1,8,1  
 #379549 Name: IRON(II) PHTHALOCYANINE  
 #408875 Name: LEAD(II) TETRAKIS(4-CUMYLPHENOXY) PHTHALOCYANINE  
 #393894 Name: VANADYL 3,10,17,24-TETRA-TERT-BUTYL-1,8,15,22-TETRAKI  
 20 #432946 Name: COPPER(II) TET  
 #441082 Name: GALLIUM(III) P  
 #423157 Name: 2,9,16,23-TETR  
 #393886 Name: COPPER(II) 3,10,17,24-TETRA-TERT-BUTYL-1,8,15,22-TETR  
 25 #418269 Name: ALUMINUM 1,8,1  
 #423165 Name: COPPER(II) 2,9  
 #430994 Name: ZINC 2,9,16,23  
 #307696 Name: COBALT(II) PHTHALOCYANINE Catalog Number  
 #432180 Name: SILICON 2,9,16  
 30 #446637 Name: ALUMINUM PHTHA  
 #253103 Name: 29H,31H-PHTHALOCYANINE, 98% Catalog Num  
 #379565 Name: LEAD(II) PHTHALOCYANINE  
 #418277 Name: ALUMINUM 2,9,1  
 #362530 Name: ALUMINUM PHTHALOCYANINE CHLORIDE Catalo  
 35 #444529 Name: ZINC 1,2,3,4,8  
 #452521 Name: IRON(III) PHTH  
 #446645 Name: COBALT(II) 1,2  
 #446653 Name: COPPER(II) 1,2  
 #448044 Name: IRON(II) 1,2,3  
 40 #386626 Name: ALUMINUM 1,4,8,11,15,18,22,25-OCTABUTOXY-29H,31H-PHTH  
 #360635 Name: NICKEL(II) PHTHALOCYANINE Catalog Number  
 #448311 Name: COPPER(II) 1,2  
 #428159 Name: SILICON(IV) PH  
 45 #386618 Name: COPPER(II) 1,4,8,11,15,18,22,25-OCTABUTOXY-29H,31H-PH  
 #287768 Name: SILICON PHTHALOCYANINE DICHLORIDE Catal  
 #408883 Name: NICKEL(II) 1,4,8,11,15,18,22,25-OCTABUTOXY-29H,31H-PH  
 50 #383813 Name: ZINC 1,4,8,11,15,18,22,25-OCTABUTOXY-29H,31H-PHTHALOC  
 #362549 Name: DILITHIUM PHTHALOCYANINE  
 #383805 Name: 1,4,8,11,15,18,22,25-OCTABUTOXY-29H,31H-PHTHALOCYANIN  
 55 #252980 Name: COPPER(II) PHTHALOCYANINE Catalog Number  
 #245356 Name: COPPER(II) PHT

An example of a traditional near-IR absorbing dye, also used for diagnostic purposes, is Aldrich #22886-9 dye, indocyanine green. Another, used as a biological stain, is Aldrich #11991-1, naphthol green B. Of all these dyes, a particularly good choice for this application are the naphthalocyanine dyes which have low water solubility but high solubility in nonpolar polymers. For example, vanadyl 5,14,23,32-tetraphenyl 2,3-naphthalocyanine [Aldrich 39,317-7 (CA 131220-68-3)] with a molecular formula weight of 1084 daltons exhibits a strong absorption peak (with a molar extinction coefficient of ~200,000 at 846nm) and high solubility in ethylene vinyl acetate (EVA) low melting polymers (such as Dupont ELVAX™ 410). This dye absorption peak matches well the emission wavelength of selected GaAlAs laser diodes. Similarly, vanadyl 2,11,20,29-tetra-tert-butyl-2,3-naphthalocyanine [CA 105011-00-5] FW1004 absorbs near IR with a narrow peak at 808nm which closely matches the emission wavelength (selected by choosing a different value of [Al]) of GaAlAs laser diodes widely used to pump solid state Nd:YAG lasers. All these naphthalocyanine dyes (Table 2) are highly soluble in EVA polymer thermoplastics and other similar thermoplastic materials. They are quite stable compounds particularly with heating to ~300°C and do not exhibit adverse photochemistry which might affect biological macromolecules in the tissue.

A table of naphthalocyanine dyes as presented in the Aldrich Catalog is presented below:

TABLE 2: NAPHTHALOCYANINE DYES (Aldrich Chemical Company)

- 1) vanadyl 5,14,23,32-tetraphenyl 2,3-naphthalocyanine  
Aldrich 39,317-7CA 131220-68-3FW1084 846nm p. 104
- 2) tin(IV) 2,3-naphthalocyanine dichloride  
Aldrich 40,651-1CA 26857-61-4 FW902 828nm p. 102
- 3) silicon(IV) 2,3-naphthalocyanine dihydroxide  
Aldrich 40,653-8CA 92396-90-2 FW775 785nm p. 94
- 4) silicon(IV) 2,3-naphthalocyanine dioctyloxide  
Aldrich 40,767-4CA 92941-50-9 FW941 798nm p. 94
- 5) 5,9,14,18,23,27,32,36-octabutoxy 2,3-naphthalocyanine  
Aldrich 41,207-4CA 105528-25-4FW1292 867nm p. 181

6) copper(II) 5,9,14,18,23,27,32,36-octabutoxy 2,3-naphthalocyanine  
Aldrich 41,528-6CA 155773-67-4FW 853nm p. 33

5 7) nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine  
Aldrich 41,885-4CA 155773-70-9FW1348 848nm p. 78

10 8) vanadyl 2,11,20,29-tetra-tert-butyl-2,3-naphthalocyanine  
Aldrich 43,296-2CA 105011-00-5FW1004 808nm p. 1524 '96

15 The interaction of laser beam Z or other activating light source is shown in Fig. 1. Most conveniently, most conventional microscopes have provision for introducing such epi-illumination to the sample. Activating light - such as laser Z - can be conveniently introduced in such light paths.

20 A variety of wavelengths of electromagnetic energy can be used in the practice of the invention provided that suitable materials are used. In particular, it is important that the transfer film 94 absorb sufficient energy (or contain one or more dyes that absorb sufficient energy) at the chosen wavelength to melt or nearly melt the thermoplastic polymer in the targeted region. For thermoplastic materials such as ethylene vinyl acetate (EVA), a wavelength of about 3 to about 10 micrometers is preferred as these materials intrinsically absorb in this range. In one embodiment, the power of the laser is used generally in the range of from about 1 mW to about 200 mW depending on the size of the target (i.e., increasing power with increasing target size). It is also preferred that the wavelengths for laser activation and film absorption be chosen outside the normal range used for microscopic imaging. Reproducible microtransfer of tissue can be obtained using a variety of infrared wavelengths from the laser.

35 Suitable lasers for use in the present invention include carbon dioxide lasers (9.6- 11  $\mu$ m wavelengths), laser diodes, tunable single frequency Ti:sapphire lasers and diode-pumped NdYAG lasers. The wavelength outputs from these lasers can preferably range from ultraviolet to infrared. A particularly desirable laser for use with the present invention is the laser diode with wavelengths between 690 and 1300 nm. In this wavelength range, conventional glass microscope optics is

40

highly transmissive and can be used to focus the laser. This is a marked improvement over laser capture microdissection designs using longer laser wavelengths (e.g., 9.6- 11  $\mu\text{m}$ ) for the carbon dioxide laser or  $\sim 3\mu\text{m}$  with the Er:YAG with laser diode pumping or- intrinsic).

Referring to Figs. 4B, an important feature of this invention may be observed. Specifically, the footprint of contact of coated collection tip T with sample A can be larger than the footprint of desired cells 88 of sample A. By the expedient of activating coated collection tip T only in the profile of the footprint of desired cells 88, precise collection can occur. Thus, not all cells within the contact area are necessarily removed. This is what is shown in Figs. 4A and 4B.

Further, and from the discussion above, it will be understood that two features can control the footprint of contact (as distinguished from the footprint of activation) of coated collection tip T with sample A. First, and as discussed above, the force of contact will in some measure control the size of the footprint of contact 96 illustrated between the broken lines of Fig. 4B.

Second, the particular shape of the convex surface can likewise control the size of the footprint of contact. For this, reference is made to Figs. 5A - 5E. It is assumed that the contact surface is substantially parallel (at least locally) to the surface of sample A.

Fig. 5A represents coated collection tip T having a right cylindrical shape. Depending upon force applied, cylindrical contact footprint 100 is either a straight line or a rectilinear patch. Given the angularity of sample collecting rod R with respect to sample A, such a surface is not desired.

Fig. 5B illustrates coated collection tip T having the profile of one half of an oblate spheroid (football shaped). Such a coated collection tip T has a roughly elliptical contact profile 102.

Fig. 5C illustrates coated collection tip T having a spherical surface. Such a coated collection tip T would have

a circular contact profile 104 - which upon reduction of the contact force would effectively reduce to a point.

Fig. 5D illustrates coated collection tip T having a faceted sphere with discrete facets 106. Such discrete facets 106 would themselves define the contact area.

Fig. 5E includes a coated collection tip T having planar facets 108 extending along the length of the cone. Again, the facets will define the area of contact with sample A.

It will be understood that once convex surface X is produced, the coating of the selectively activatable adhesive on the contact surface can be made in any manner desired. For example, dipping convex surface X into a solution which upon drying or curing forms the required coating will suffice for the practice of this invention. Alternatively, forming the coating in a specially contoured mould cavity can be done using molten material attached to the outside of the convex surface, which then is placed inside the cavity.

Referring to Fig. 6A, dip coating sample collecting rod R with activatable adhesive is done by mounting it in a suitable schematically shown rotating device 112, inserting the rod vertically with rotation down into heated solution 114 of 20% weight/volume EVA (DuPont Elvax 410) in tetrachloroethylene so the tip is immersed to the desired depth of coating, and withdrawing the rod. As shown in Fig. 6B, sample collecting rod R is then moved to a horizontal position with continued rotation and the solvent allowed to evaporate. A draft of air, such as conducting the operation in a hood, is desirable. A suitable angle of positioning the rod other than exactly horizontal may be desired for best distribution of the coating, and it may be desirable to remove solution from the end of the rod before evaporation. The temperature of the solution can be varied and, as an example, 50 degrees C can be used. Multiple coatings can be made to obtain a desired thickness, and two or three coats, made with evaporation between dipping, are done. Coating 94 on sample collecting rod R results.

As shown methods of coating include dipping the rod (see Figs. 6A and 6B), spraying at nozzle 116 the solution on the



rod (see Fig. 7), and adding controlled volumes of solution to the rod with micropipet 118 (see Fig. 8). Each of these methods of coating the rod with solution are followed by evaporation of the solvent, and with each method, rotation of the rod during addition of the solution and evaporation is desirable by schematically shown rotating device 112. It is found desirable in many instances to impress the coated rod R within a specially contoured mould cavity 111 whose concave contour will produce the desired convex surface. The coating or the mould or both should be heated to accomplish this shaping operation.

Solutions of hot-melt adhesive can be made in a variety of solvents. For the ethylene vinyl acetate (EVA) used, toluene (109-88-3) and a variety of chlorinated hydrocarbon solvents can be used. Examples of chlorinated solvents which can be used are methylene chloride (75-09-2), carbon tetrachloride (56-23-5), chloroform, (67-66-3), tetrachloroethane (630-20-6), tetrachloroethylene (127-18-4), trichloroethylene (79-01-6) and 1,1,1-trichloroethane (71-55-6). It would be expected that other solvents used for polymer and vinyl solutions would be suitable. The choice of solvent is dictated by considerations of safety, solvent effectiveness, and volatility. The latter is particularly important, in matching volatility to the effectiveness of the process and characteristics of the coating such as evenness, smoothness, depth of coating and rate of drying.

Solutions of EVA can be made in various concentrations and higher concentrations, above 15% or 20% weight/volume for some solvents, must be heated above room temperature to remain liquid. The optimum concentration depends on the coating method, for example spraying works best with lower concentrations and higher concentrations are best for dipping. An advantage of dipping with higher concentrations of solution, which are heated to remain liquid, is that the cooling of the coating on the rod results in deposition of a thick film.

Rotation of the rod during coating and evaporation is desirable to get best distribution of the coating.

Film coatings of a few microns (micrometers) to 100 or more microns (0.004 inches) can be obtained by these methods. After drying of the solvent, the coating can be smoothed by heat treatment or shaping in a mold.

In the following claims we utilize the words "convex." It is to be understood by this designation that we include all surfaces which place a central portion in contact with the slide and film and have peripheral surfaces away from contact with the slide. It will be understood that at least part of the surface - whether in contact with the slide or not in contact with the slide - impresses the selectively activated surface onto the sample. Upon activation this produces adhesive regions. Thus, the adhesive regions are bordered by either non-activated or selectively activated surfaces which are away from that contact. Thus, upon reorientation, the peripheral regions can be utilized for activation and collection.

We therefore include convex surfaces which are spheres, oblate spheroids (football shapes), cones, frustum shapes (truncated cones), cylinders, and the like.

It will further be understood that all of the above surfaces can be faceted. We therefore include such faceted surfaces placed on "convex" shapes.

WHAT IS CLAIMED IS:

- 1           1.    A method of direct extraction of cellular  
2 material from a tissue sample which comprises:  
3           providing a tissue sample;  
4           providing a selectively activated surface which can be  
5 activated to provide selectively activated regions thereof  
6 with adhesive properties;  
7           providing a convex surface;  
8           identifying at least one portion of the tissue sample  
9 which is to be extracted;  
10          juxtaposing at least one portion of the tissue sample  
11 with the convex surface;  
12          selectively activating the portion of the selectively  
13 activated surface to form an adhesive transfer surface;  
14          contacting the tissue sample with the adhesive transfer  
15 surface; and  
16          separating the adhesive transfer surface from the tissue  
17 sample while maintaining adhesion with the at least one  
18 portion of the tissue sample so that the at least one portion  
19 of the tissue sample is extracted from a remaining portion of  
20 the tissue sample and attached to the adhesive transfer  
21 surface.
- 1           2.    A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the contacting step occurs before the selectively activating  
4 step.
- 1           3.    A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the contacting step occurs after the selectively activating  
4 step.
- 1           4.    A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the selectively activated surface is attached to the convex  
4 surface.

1           5. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the convex surface is cylindrical.

1           6. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 5, wherein  
3 the cylindrical surface makes a line contact with the tissue  
4 sample.

1           7. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the convex surface is conical.

1           8. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 7 and wherein  
3 the conical surface is a frustum of a cone.

1           9. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 7 and wherein  
3 the convex surface makes a line contact with the tissue  
4 sample.

1           10. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the convex surface is provided with facets.

1           11. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 10 and  
3 wherein only one facet at a time comes in contact with the  
4 tissue sample.

1           12. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the convex surface is located at the extremity of a rod.

1           13. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the convex surface is spherical.

1           14. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 13 and  
3 wherein the spherical surface has a circular contact with the  
4 tissue sample.

1           15. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 1 and wherein  
3 the convex surface is an oblate spheroid.

1           16. A method of direct extraction of cellular  
2 material from a tissue sample according to claim 15 and  
3 wherein the oblate spheroid contacts the tissue sample at an  
4 elliptical patch.

1           17. A method of direct extraction of desired  
2 cellular material from a tissue sample having diverse cellular  
3 material including the steps of:

4           a. providing a tissue sample having dispersed  
5 desired cellular material scattered throughout the tissue  
6 sample;

7           b. providing a selectively activated convex  
8 surface which can be activated to provide selective regions  
9 thereof with adhesive properties;

10          c. identifying at least one portion of the  
11 tissue sample with desired cellular material which is to be  
12 extracted;

13          d. contacting the at least one portion of the  
14 tissue sample with the selectively activated convex surface;

15          e. selectively activating a region of the  
16 selectively activated convex surface which is in contact with  
17 the desired cellular material of the tissue sample to form an  
18 adhesion region that selectively adheres to the desired  
19 cellular material;

20          f. separating the adhesion region from the  
21 tissue sample while maintaining adhesion between the adhesion  
22 region and the desired cellular material of the tissue sample  
23 so that at least a part of the at least one portion of the

24 tissue sample is extracted from a remaining portion of the  
25 tissue sample;  
26 g. reorienting the convex surface with  
27 respect to the tissue sample; and,  
28 h. repeating steps c., d., e., and f. to  
29 place desired cellular material on a different region of the  
30 selectively activated convex surface.

1 18. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 17  
3 and wherein:  
4 step h. includes repeating steps c., d., e.,  
5 f., and g. so as to pool desired cellular material on  
6 different regions of the selectively activated convex surface.

1 19. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 17  
3 and wherein:  
4 the step of reorienting the convex surface includes  
5 rolling the convex surface over the tissue sample.

1 20. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 17  
3 and wherein:  
4 the step of reorienting the convex surface includes  
5 lifting the convex surface away from the tissue sample and  
6 then reorienting the convex surface.

1 21. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 20  
3 and wherein:  
4 the step of reorienting includes rotating the convex  
5 surface.

1 22. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 20  
3 and wherein:

4           the repeating step occurs in separated portions of  
5 the tissue sample on the same slide.

1           23. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 20  
3 and wherein:

4           the repeating step occurs in separated portions of  
5 tissue samples on different slides.

1           24. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 20  
3 including the steps of:

4           inspecting the desired cellular material on the  
5 convex surface; and,

6           detaching and analyzing at least some of the  
7 desired cellular material from the convex surface after the  
8 viewing step.

1           25. A method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 20  
3 including the steps of:

4           inspecting the transferred cellular material on  
5 the convex surface; and,

6           encapsulating any undesired cellular material  
7 which may have transferred to the convex surface to prevent  
8 its subsequent analysis.

1           26. In a method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 20  
3 and wherein:

4           any undesired cellular material on the convex  
5 surface is inactivated by radiation or heat.

1           27. In a method of direct extraction of desired  
2 cellular material from a tissue sample according to claim 18  
3 and wherein:

4           the desired cells as collected on the convex surface  
5 are inspected.

1                   28. In combination with a microscope having  
2                   a stage for holding a slide with a tissue sample  
3 thereon;  
4                   a light source and condenser for illuminating the  
5 tissue sample; and,  
6                   an objective/eyepiece combination for examining  
7 viewed portions of the tissue sample;  
8                   the improvement to the microscope comprises in  
9 combination:  
10                  a selectively activated convex surface which can be  
11 activated to provide selective regions thereof with adhesive  
12 properties;  
13                  means for moving the selectively activated convex  
14 surface in and out of contact with the viewed portions of the  
15 tissue sample;  
16                  means for selectively activating portions of the  
17 selectively activated convex surface in contact with the  
18 tissue sample to form adhesion regions whereby when the  
19 activated convex surface is moved out of contact with the  
20 tissue sample the adhesion region of the at least part of the  
21 viewed portions of the tissue sample adheres to the  
22 selectively activated convex surface; and,  
23                  means for reorienting the selectively activated  
24 convex surface whereby portions of the selectively activated  
25 convex surface which have not been selectively activated are  
26 exposed for contact with the tissue sample.

1                   29. The combination with a microscope according to  
2 claim 28 and wherein:  
3                   the means for moving the selectively activated  
4 convex surface in and out of contact with the viewed portions  
5 of the tissue sample includes means for moving the convex  
6 surface away from a view path from the tissue sample to the  
7 objective/eye piece combination.

1                   30. The combination with a microscope according to  
2 claim 28 wherein:  
3                   the convex surface is transparent.



1           31. The combination with a microscope according to  
2 claim 28 wherein:

3           the selectively activated convex surface is located  
4 at an extremity of a rod;

5           the means for moving the selectively activated  
6 convex surface in and out of contact with the viewed portions  
7 of the tissue sample includes a pivot attached to the rod  
8 remote from the selectively activated convex surface for  
9 pivoting the rod into and out of a light path from the tissue  
10 sample to the objective/eye piece combination for viewing the  
11 tissue sample; and,

12           the means for reorienting the selective activated  
13 convex surface includes means for rotating the rod.

1           32. The combination with a microscope according to  
2 claim 28 wherein:

3           the means for moving the selectively activated  
4 convex surface in and out of contact with the viewed portions  
5 of the tissue sample includes means for "contacting the viewed  
6 portions of the tissue sample with a measured pre-load force.

1           33. The combination with a microscope according to  
2 claim 28 wherein:

3           the selectively activated convex surface is located  
4 at an extremity of a rod; and,

5           the rod with its selectively activated convex  
6 surface is interchangeable with other similar rods with a  
7 selectively activated convex surface.

1           34. An attachment for a microscope having a stage  
2 for holding a slide with a tissue sample thereon, a light  
3 source and condenser for illuminating the tissue sample, and  
4 an objective/eyepiece combination for examining viewed  
5 portions of the tissue sample;

6           the attachment comprising in combination:

7           a selectively activated convex surface which can be  
8 activated to provide selective regions thereof with adhesive  
9 properties;

10 means for moving the selectively activated convex  
11 surface in and out of contact with the viewed portions of the  
12 tissue sample;

13 means for selectively activating portions of the  
14 selectively activated convex surface in contact with the  
15 tissue sample to form an adhesion region whereby when the  
16 adhesion region is out of contact with the tissue sample at  
17 least a part of the viewed portions of the tissue sample  
18 adhere to the selectively activated convex surface; and,

19 means for reorienting the selectively activated  
20 convex surface whereby portions of the selectively activated  
21 convex surface which have not been activated are exposed for  
22 contact with the tissue sample.

1 35. In an apparatus for laser capture  
2 microdissection, a contact surface comprising:

3 a convex surface;

4 a rod with the convex surface mounted to an  
5 extremity of the rod; and,

6 a selectively activated coating placed over the  
7 convex surface.

1 36. The apparatus for laser capture microdissection  
2 according to claim 35 wherein:

3 the convex surface is spherical.

1 37. The apparatus for laser capture microdissection  
2 according to claim 35 wherein:

3 the convex surface is faceted.

1 38. The apparatus for laser capture microdissection  
2 according to claim 35 wherein:

3 the convex surface is cylindrical.

1 39. The apparatus for laser capture microdissection  
2 according to claim 35 wherein:

3 the convex surface has the profile of frustum.

1           40. The apparatus for laser capture microdissection  
2 according to claim 35 wherein:  
3           the rod and convex surface are transparent.

1           41. In an apparatus for laser capture  
2 microdissection, a contact surface and vial comprising:  
3           a convex surface;  
4           a selectively activated coating placed over the  
5 convex surface;  
6           a vial having a dimension for permitting the convex  
7 surface to be placed into the vial; and,  
8           a fluid in the vial for liberating at least a part  
9 of a tissue sample adhered to the selectively activated convex  
10 surface.

1           42. A method of producing a convex surface for  
2 laser capture microdissection comprising the steps of:  
3           providing a rod with a convex surface;  
4           coating the rod at the convex surface with an  
5 activatable adhesive dissolved in a solvent; and,  
6           dissolving solvent to leave the activatable adhesive  
7 solidified on the rod at the convex surface.

1           43. A method of producing a convex surface for  
2 laser capture microdissection according to claim 42 and  
3 comprising the further steps of:  
4           coating includes dipping the rod at the convex  
5 surface in the activatable adhesive dissolved in a solvent.

1           44. A method of producing a convex surface for  
2 laser capture microdissection according to claim 42 and  
3 comprising the further steps of:  
4           coating includes spraying the rod at the convex  
5 surface with the activatable adhesive dissolved in a solvent.

1           45. A method of producing a convex surface for  
2 laser capture microdissection according to claim 42 and  
3 comprising the further steps of:

4           coating includes applying a measured amount of  
5    activatable adhesive in a solvent to the rod at the convex  
6    surface.

1           46. A method of producing a convex surface for  
2    laser capture microdissection according to claim 42 and  
3    comprising the further steps of:

4           coating includes impressing the activatable adhesive  
5    in a concave mould to impart the desired convex shape.

**AMENDED CLAIMS**

[received by the International Bureau on 9 December 1998 (09.12.98);  
original claims 1-46 replaced by amended claims 1-27 (5 pages)]

**WHAT IS CLAIMED IS:**

1. A method of direct extraction of cellular material from a tissue sample which comprises:  
providing a tissue sample;  
providing a convex surface  
adhering a selectively activatable layer to the convex surface to provide a selectively activatable convex surface, the selectively activatable convex surface capable of being locally activated to provide an adhesive transfer surface;  
identifying at least one portion of the tissue sample which is to be extracted;  
juxtaposing at least one portion of the tissue sample with the selectively activatable convex surface;  
selectively activating a portion of the selectively activatable convex surface to form the adhesive transfer surface;  
contacting the tissue sample with the adhesive transfer surface; and  
separating the adhesive transfer surface from the tissue sample while maintaining adhesion with the at least one portion of the tissue sample so that the at least one portion of the tissue sample is extracted from a remaining portion of the tissue sample and attached to the adhesive transfer surface.

2. A method of direct extraction of cellular material from a tissue sample according to claim 1 and wherein the contacting step occurs before the selectively activating step.

3. A method of direct extraction of cellular material from a tissue sample according to claim 1 and wherein the contacting step occurs after the selectively activating step.

4. A method of direct extraction of cellular material from a tissue sample according to claim 1 and wherein the tissue sample is mounted to a slide; and,  
the selectively activated surface is activated through the slide and tissue sample.

5. A method of direct extraction of cellular material from a tissue sample according to claim 1 and wherein the convex surface is cylindrical.

1                   6.     A method of direct extraction of cellular material from a tissue sample  
2 according to claim 5, wherein the cylindrical surface makes a line contact with the tissue  
3 sample.

1                   7.     A method of direct extraction of cellular material from a tissue sample  
2 according to claim 1 and wherein the convex surface is conical.

1                   8.     A method of direct extraction of cellular material from a tissue sample  
2 according to claim 7 and wherein the conical surface is a frustum of a cone.

1                   9.     A method of direct extraction of cellular material from a tissue sample  
2 according to claim 7 and wherein the convex surface makes a line contact with the tissue  
3 sample.

1                   10.    A method of direct extraction of cellular material from a tissue sample  
2 according to claim 1 and wherein the convex surface is provided with facets.

1                   11.    A method of direct extraction of cellular material from a tissue sample  
2 according to claim 10 and wherein only one facet at a time comes in contact with the tissue  
3 sample.

1                   12.    A method of direct extraction of cellular material from a tissue sample  
2 according to claim 1 and wherein the convex surface is located at the extremity of a rod.

1                   13.    A method of direct extraction of cellular material from a tissue sample  
2 according to claim 1 and wherein the convex surface is spherical.

1                   14.    A method of direct extraction of cellular material from a tissue sample  
2 according to claim 13 and wherein the spherical surface has a circular contact with the tissue  
3 sample.

1                   15.    A method of direct extraction of cellular material from a tissue sample  
2 according to claim 1 and wherein the convex surface is an oblate spheroid.

1                   16.    A method of direct extraction of cellular material from a tissue sample  
2 according to claim 15 and wherein the oblate spheroid contacts the tissue sample at an  
3 elliptical patch.

- 1                   17.    A method of direct extraction of desired cellular material from a tissue  
2 sample having diverse cellular material including the steps of:
- 3                   a.     providing a tissue sample having dispersed desired cellular  
4 material scattered throughout the tissue sample;
- 5                   b.     providing a selectively activatable convex surface which can be  
6 activated to provide selective regions thereof with adhesive properties;
- 7                   c.     identifying at least one portion of the tissue sample with desired  
8 cellular material which is to be extracted;
- 9                   d.     juxtaposing the at least one portion of the tissue sample with  
10 the selectively activatable convex surface;
- 11                  e.     selectively activating and contacting a region of the selectively  
12 activatable convex surface with the desired cellular material of the tissue sample to form an  
13 adhesion region that selectively adheres to the desired cellular material;
- 14                  f.     separating the adhesion region from the tissue sample while  
15 maintaining adhesion between the adhesion region and the desired cellular material of the  
16 tissue sample so that at least a part of the at least one portion of the tissue sample is extracted  
17 from a remaining portion of the tissue sample;
- 18                  g.     reorienting the convex surface with respect to the tissue  
19 sample; and,
- 20                  h.     repeating steps c., d., e., and f. to place desired cellular material  
21 on a different region of the selectively activatable convex surface.

- 1                   18.    A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 17 and wherein:
- 3                   step h. includes repeating steps c., d., e., f., and g. so as to accumulate  
4 desired cellular material from spatially disbursed regions onto different but closely spaced  
5 regions of the selectively activatable convex surface.

- 1                   19.    A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 17 and wherein:
- 3                   the step of reorienting the convex surface includes rolling the convex surface  
4 over the tissue sample.

1                   20.     A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 17 and wherein:

3                   the step of reorienting the convex surface includes lifting the convex surface  
4 away from the tissue sample and then reorienting the convex surface.

1                   21.     A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 20 and wherein:

3                   the step of reorienting includes rotating the convex surface.

1                   22.     A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 20 and wherein:

3                   the repeating step occurs in separated portions of the tissue sample on the  
4 same slide.

1                   23.     A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 20 and wherein:

3                   the repeating step occurs in separated portions of tissue samples on different  
4 slides.

1                   24.     A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 20 including the steps of:

3                   inspecting the desired cellular material on the convex surface; and,  
4                   detaching and analyzing at least some of the desired cellular material  
5 from the convex surface after the viewing step.

1                   25.     A method of direct extraction of desired cellular material from a tissue  
2 sample according to claim 20 including the steps of:

3                   inspecting the transferred cellular material on the convex surface; and,  
4                   encapsulating any undesired cellular material which may have  
5 transferred to the convex surface to prevent its subsequent analysis.

1                   26.     In a method of direct extraction of desired cellular material from a  
2 tissue sample according to claim 20 and wherein:

3                   any undesired cellular material on the convex surface is inactivated by  
4 radiation or heat.



- 1                    27.    In a method of direct extraction of desired cellular material from a  
2   tissue sample according to claim 18 and wherein:  
3                    the desired cells as collected on the convex surface are inspected.

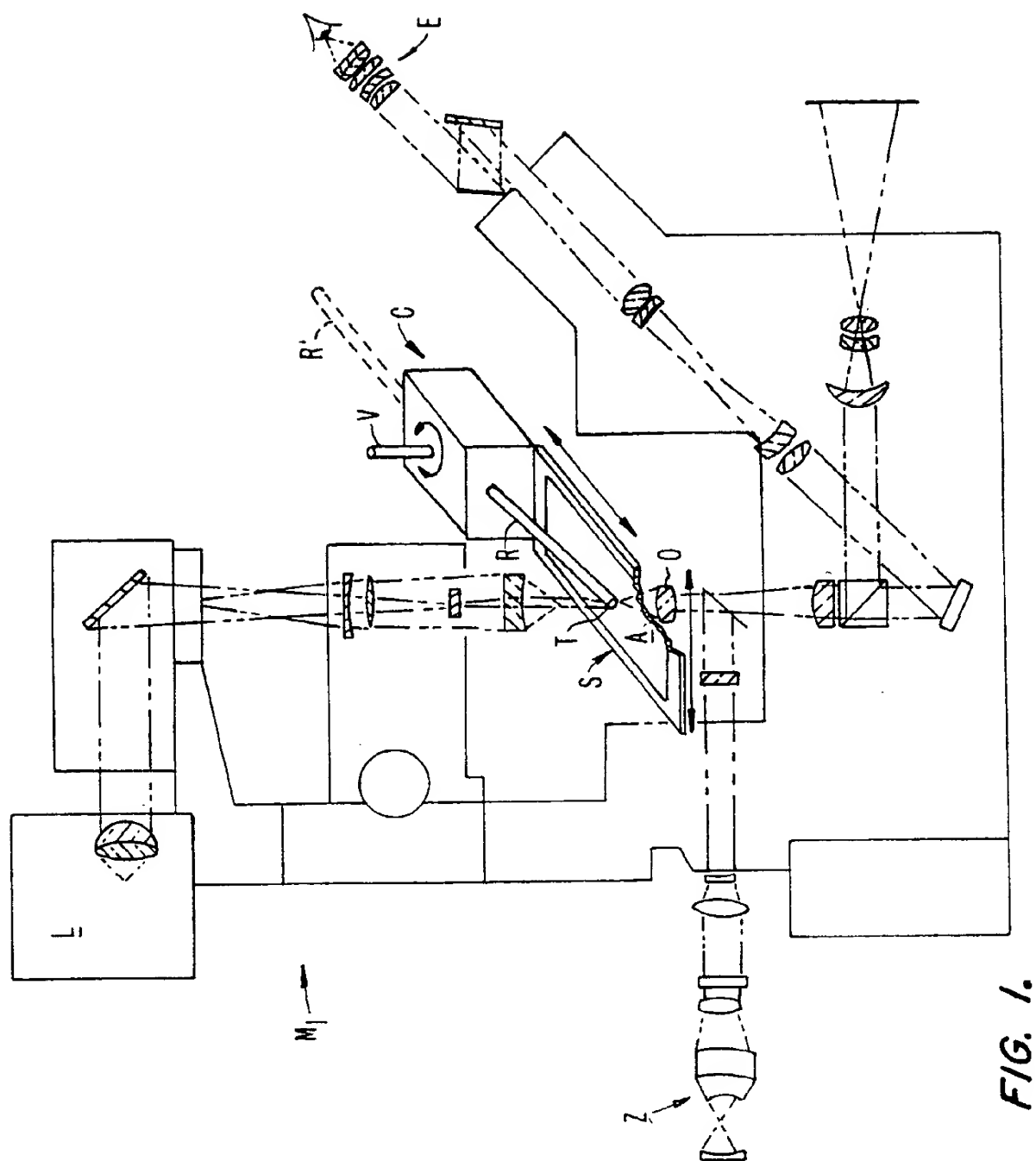
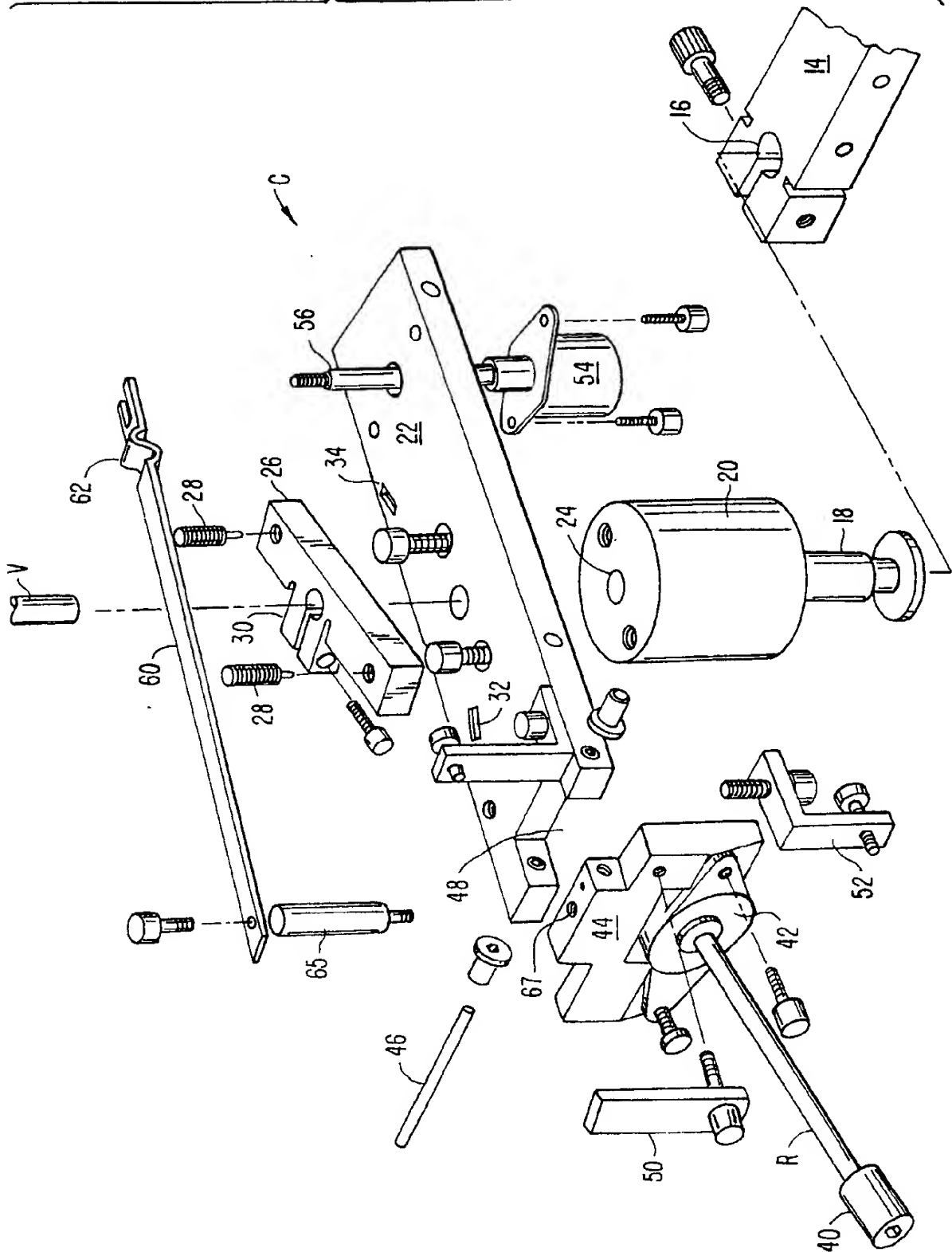


FIG. 1.

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FIG. 2A.



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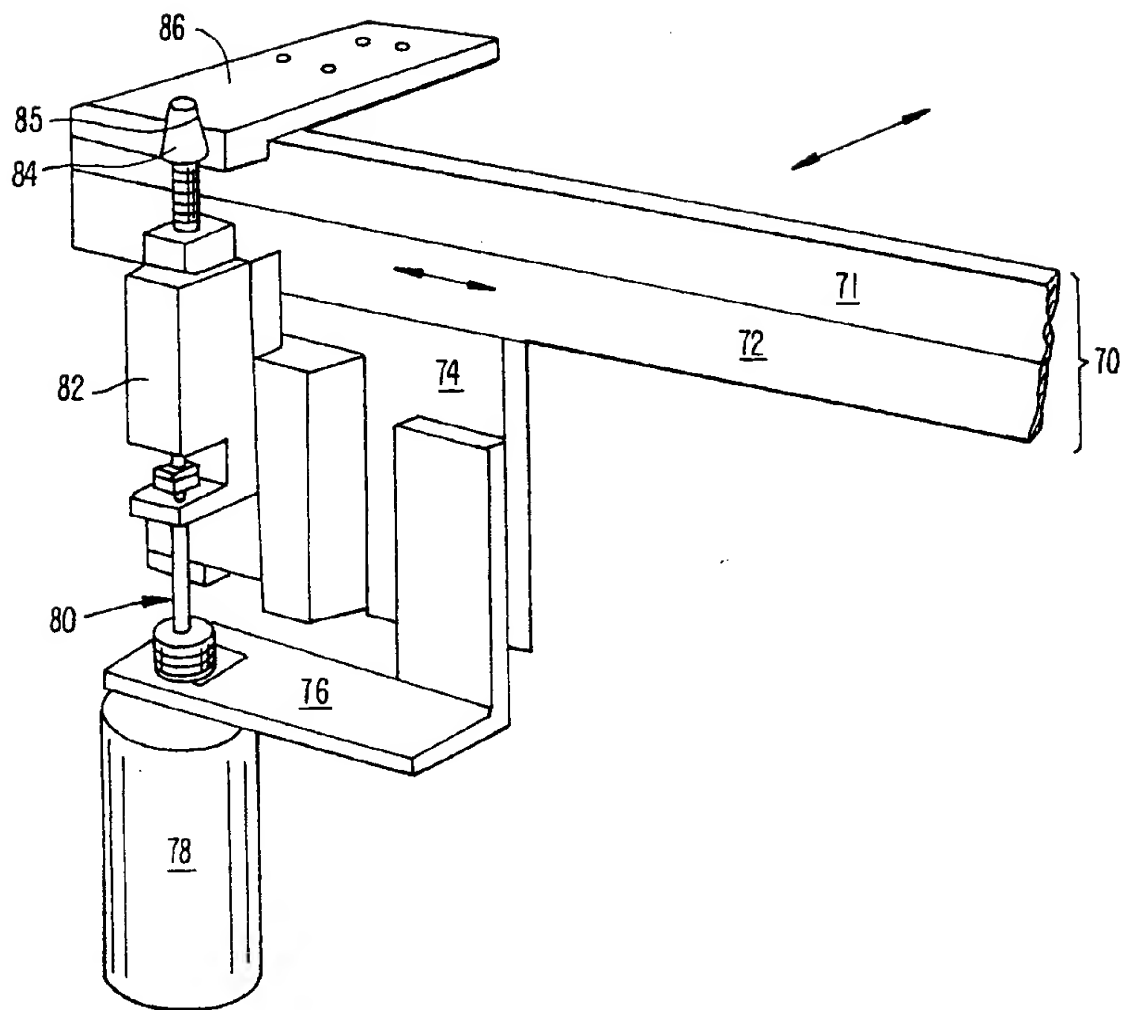


FIG. 2B.

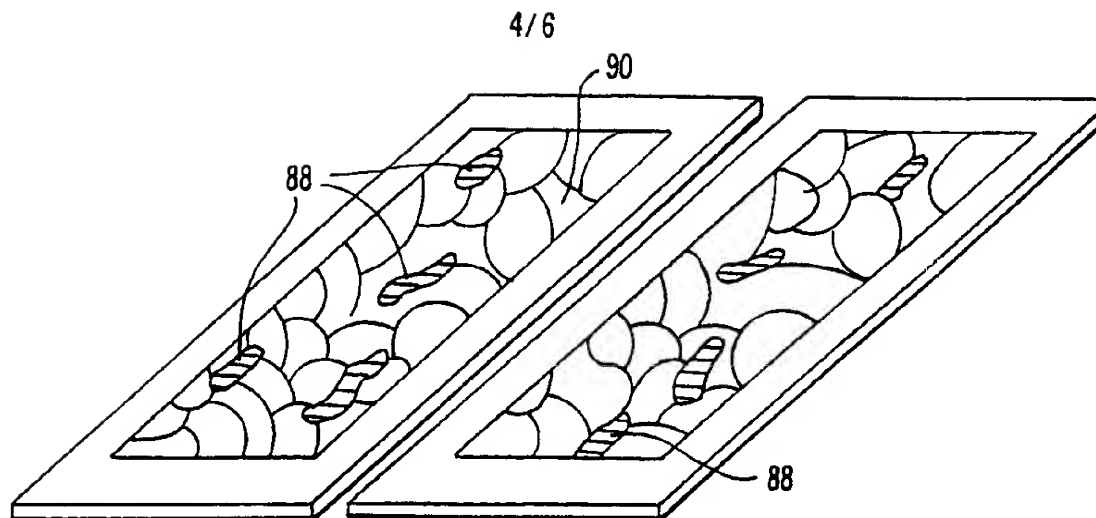


FIG. 3.

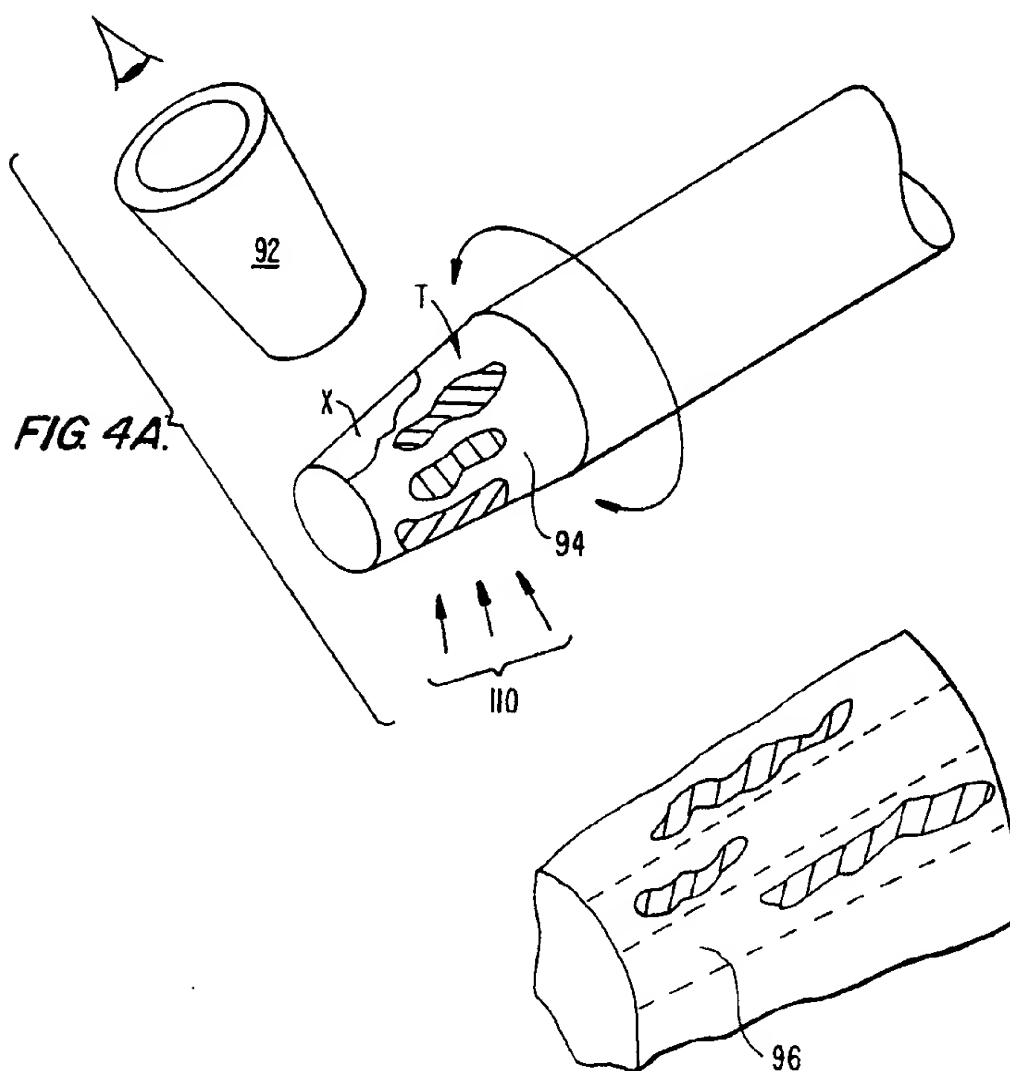
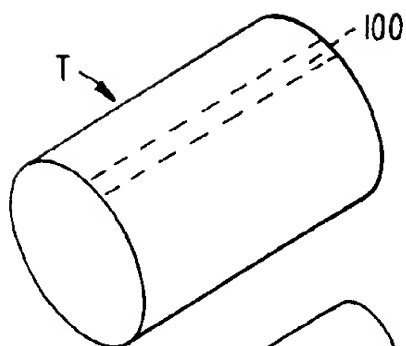


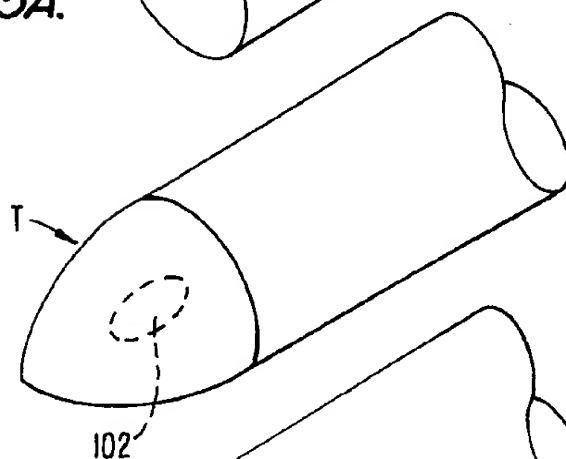
FIG. 4B.

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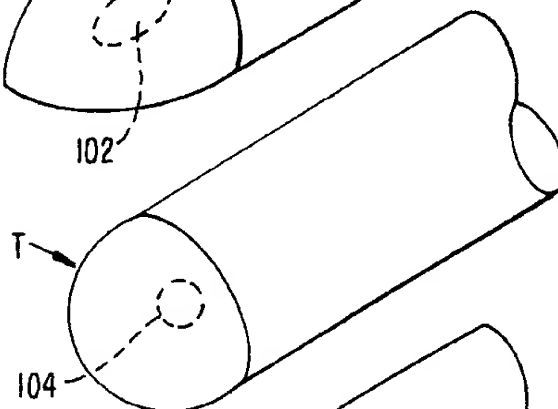
**FIG. 5A.**



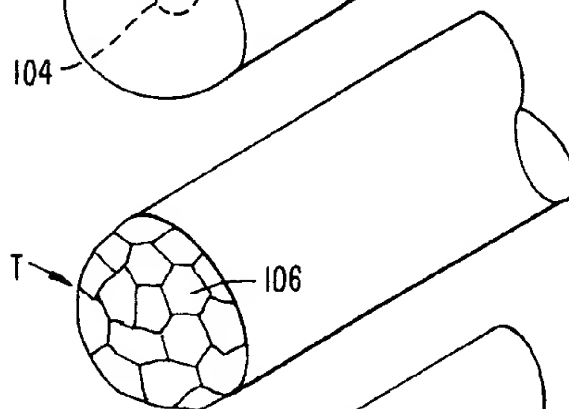
**FIG. 5B.**



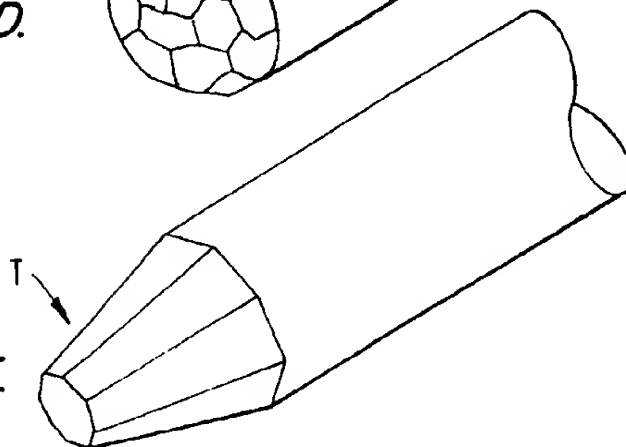
**FIG. 5C.**



**FIG. 5D.**



**FIG. 5E.**



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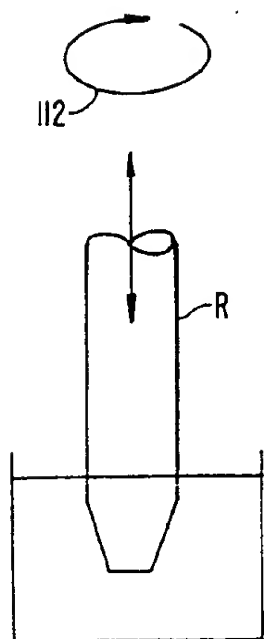


FIG. 6A.

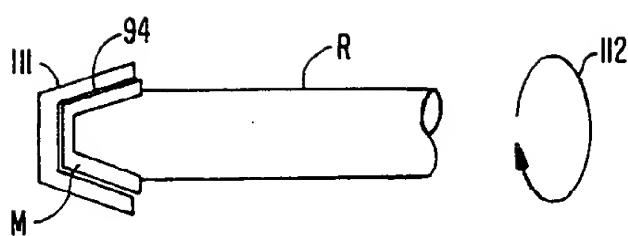


FIG. 6B.

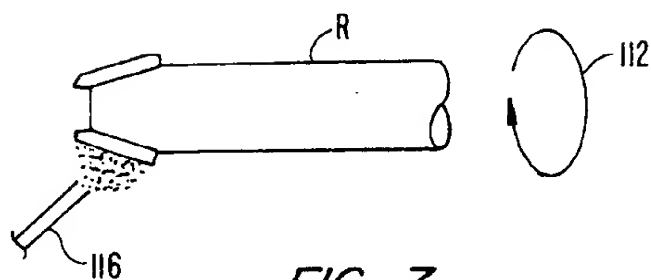


FIG. 7.

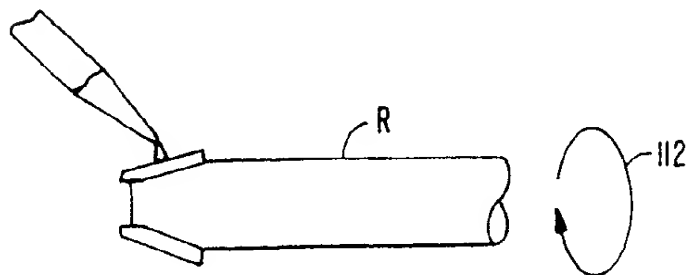


FIG. 8.

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/13318

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 G01N1/28 G02B21/34

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N G02B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 13838 A (US HEALTH; LIOTTA LANCE A (US); EMMERT BUCK MICHAEL (US); KRIZMAN) 17 April 1997	1, 3, 4, 7, 8, 10-12, 35, 37, 39, 41-43, 45
A	see page 11, line 10 - page 12, line 34; figures 2A-C see page 18, line 21 - page 30, line 33	17, 28, 34
A	EP 0 539 888 A (SHIMADZU CORP) 5 May 1993 see column 7, line 36 - column 8, line 19	1, 17, 28, 34, 41-43
A	EMMERT-BUCK M R ET AL: "LASER CAPTURE MICRODISSECTION" SCIENCE, vol. 274, no. 5289, 8 November 1996, pages 998-1001, XP000644727	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

6 October 1998

Date of mailing of the international search report

16/10/1998

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Hodson, M



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/13318

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9713838 A	17-04-1997	AU 7663396 A EP 0862612 A	30-04-1997 09-09-1998
EP 0539888 A	05-05-1993	JP 5127099 A DE 69210753 D DE 69210753 T US 5348883 A	25-05-1993 20-06-1996 02-10-1996 20-09-1994